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Ipl1/Aurora B kinase coordinates synaptonemal complex disassembly with cell cycle progression and crossover formation in budding yeast meiosis

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Several protein kinases collaborate to orchestrate and integrate cellular and chromosomal events at the G2/M transition in both mitotic and meiotic cells. During the G2/M transition in meiosis, this includes the completion of crossover recombination, spindle formation, and synaptonemal complex (SC) breakdown. We identified Ipl1/Aurora B kinase as the main regulator of SC disassembly. Mutants lacking Ipl1 or its kinase activity assemble SCs with normal timing, but fail to dissociate the central element component Zip1, as well as its binding partner, Smt3/SUMO, from chromosomes in a timely fashion. Moreover, lack of Ipl1 activity causes delayed SC disassembly in a *cdc5* as well as a *CDC5-inducible ndt80* mutant. Crossover levels in the *ipl1* mutant are similar to those observed in wild type, indicating that full SC disassembly is not a prerequisite for joint molecule resolution and subsequent crossover formation. Moreover, expression of meiosis I and meiosis II-specific B-type cyclins occur normally in *ipl1* mutants, despite delayed formation of anaphase I spindles. These observations suggest that Ipl1 coordinates changes to meiotic chromosome structure with resolution of crossovers and cell cycle progression at the end of meiotic prophase.

[**Keywords:** Meiosis; synaptonemal complex; chromosome structure; Ipl1/Aurora B kinase; Cdc5/Polo-like kinase]

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The meiotic cell cycle consists of a single round of DNA replication followed by two consecutive nuclear divisions, named meiosis I and meiosis II. At the first meiotic division, homologous chromosomes (homologs) segregate to opposite spindle poles, mediated in part by chiasmata that provide a physical link between the homologs. At meiosis II, sister chromatids separate, analogous to a mitotic division, resulting in the formation of haploid gametes (Petronczki et al. 2003).

Several modifications to chromosome structure and behavior precede meiosis I. Sister chromatids shorten and form a "core" from which loops of chromatin emanate and on which axial element components assemble during leptotene. As the axial elements of the two homologs become stably juxtaposed (~100 nm) by the deposition of

transverse filament(s) at zygotene, they form the lateral elements of the morphologically conserved synaptonemal complex (SC) (Roeder 1997; Zickler and Kleckner 1999). By pachytene, homologous chromosomes are synapsed along their entire length and the chromosomes are highly compacted. Upon pachytene exit, the chromatin decondenses and the SC is disassembled as cells enter the diffuse stage. At diplotene, most of the SC has been removed, centrosomes are separated, and chiasmata become cytologically visible. Eventually, metaphase I spindles are formed as cells prepare for the first nuclear division.

Budding yeast has a single transverse filament protein, termed Zip1 (Sym et al. 1993; Sym and Roeder 1995)—the structural equivalent of SYCP1/SYP-1 in mammals and nematodes, c(3)g in *Drosophila*, and ZYP-1 in *Arabidopsis* (Colaiacono 2006; Bhalla and Dernburg 2008). Initiation of Zip1 deposition along chromosome cores occurs from centromeres (Tsubouchi and Roeder 2005; Tsubouchi et al. 2008) as well as from future sites of crossover formation (the precursors to chiasmata) along chromosome arms (Roeder 1997; Henderson and Keeney 2005).

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At centromeres, Zip1 protects meiotic cells from the formation of aneuploid spores, since *zip1* mutants show increased crossover formation near centromeres (Chen et al. 2008) and such crossovers are associated with the precocious separation of sister chromatids at meiosis I (Rockmill et al. 2006). Furthermore, Zip1 also promotes centromere tethering, and thereby accurate segregation of chromosome pairs that fail to cross over (L Newnham, P Jordan, B Rockmill, S Roeder, and E Hoffmann, in prep.).

Along chromosome arms, crossover recombination is initiated from a subset of meiotic recombination events induced by the introduction of double-strand breaks (DSBs). In budding yeast, the differentiation of DSBs to yield crossovers or noncrossovers is imposed prior to or concomitant with strand exchange (Allers and Lichten 2001; Hunter and Kleckner 2001). Moreover, crossovers are preceded by specific DSB repair intermediates, including single-end invasions (SEIs) and joint molecules (Schwacha and Kleckner 1995; Allers and Lichten 2001; Hunter and Kleckner 2001). Consistent with SC components being important for the initiation of crossover recombination, SC mutants show a decrease in the levels of SEIs (Borner et al. 2004) as well as subsequent intermediates and crossover products (Sym et al. 1993; Storlazzi et al. 1996; Borner et al. 2004). However, the role of Zip1 at sites of crossovers is unclear. End-to-end polymerization of Zip1 along chromosomes is not required to promote crossovers in certain mutant backgrounds (Storlazzi et al. 1996; Hooker and Roeder 2006), suggesting that SC nucleation may be sufficient to facilitate the molecular transitions during crossover initiation (Storlazzi et al. 1996).

Zip1 has been proposed to bind sumoylated proteins along the lateral elements (Cheng et al. 2006; Hooker and Roeder 2006). As recombination proceeds and linear Zip1-SUMO stretches are formed, chromosomes progressively condense, culminating in highly compacted "worm-like" structures by pachytene. Both DSB repair and SC formation are monitored by checkpoints that prevent pachytene exit, the first step in the G2/M progression, until these processes are complete (Roeder and Bailis 2000; Wu and Burgess 2006). Pachytene exit is regulated by the Ndt80 transcription factor that controls the expression of ~200 genes leading to cell cycle progression (Xu et al. 1995; Chu and Herskowitz 1998; Chu et al. 1998).

The first cytological hallmark of pachytene exit is the disassembly of the SC. We showed recently that Zip1 dissociates from chromosome arms, but remains at centromeres, after SC disassembly. By diplotene, Zip1 foci colocalize with Ctf19-13Myc, a centromere marker, suggesting that Zip1 is retained specifically at centromeres. Zip1 is also visible on metaphase I spindles, then eventually localizes to the poles of anaphase I spindles together with Ctf19-13Myc (L Newnham, P Jordan, B Rockmill, S Roeder, and E Hoffmann, in prep.). Thus, Zip1 likely remains associated with centromeric regions after SC disassembly. It is unknown what facilitates the removal of Zip1 from arm regions and how Zip1 is protected (or reloaded) at centromeres at the G2/M transition.

SC disassembly is coordinated with other chromosomal and cellular events, including the appearance of crossover products; the separation of the microtubule organizing centers, known as spindle pole bodies (SPBs) in budding yeast; and modifications to kinetochores that ensure that sister kinetochores orient toward the same spindle pole at meiosis I (Toth et al. 2000). In budding yeast, *ndt80* mutants arrest with unseparated SPBs, unresolved joint molecules, and full SCs (Xu et al. 1995; Allers and Lichten 2001). Timely SC disassembly as well as joint molecule resolution can be rescued by the expression of a single gene: Cdc5/Polo-like kinase (Sourirajan and Lichten 2008). Clb1, Clb3, Clb4, and Clb6, B-type cyclin components of mitotic cyclin-dependent kinase (CDK-M), which are also regulated by Ndt80, are presumably still required for SPB separation and entry to M phase, because these events are still inhibited (Sourirajan and Lichten 2008).

The mechanism of coordination between joint molecule resolution into crossovers and SC disassembly remains unclear. *cdc5* mutants fail to resolve the majority of joint molecules but show a less pronounced SC disassembly defect (Clyne et al. 2003). Thus, although Cdc5 is sufficient to mediate SC disassembly in *ndt80Δ* mutants (Sourirajan and Lichten 2008), it is unclear whether it is required to do so in normal meiosis.

Unlike Cdc5 and CDK1-M, Ipl1/Aurora B kinase, a chromosomal passenger protein, is not regulated by Ndt80 and is expressed maximally between 3 and 6 h in meiosis, at the G2/M transition (Chu 1998; Monje-Casas et al. 2007). These proteins regulate kinetochore-microtubule attachments, spindle formation, and cytokinesis (Ruchaud et al. 2007; Kelly and Funabiki 2009), as well as chromosome dynamics at the G2/M transition (Hsu et al. 2000). Characterization of Ipl1 activity during meiosis has focused on its role in chromosome segregation. Together with the monopolin complex that ensures the cosegregation of sister kinetochores to the same spindle pole at meiosis I (Toth et al. 2000), Ipl1 promotes accurate homolog segregation at the first meiotic division (Monje-Casas et al. 2007). In addition, Ipl1 also ensures the retention of protein phosphatase 2A (PP2A) at kinetochores. PP2A protects Sgo1 and thereby centromeric cohesin, which must be retained until meiosis II to prevent precocious sister chromatid separation at meiosis I (Riedel et al. 2006; Tang et al. 2006; Yu and Koshland 2007).

Ipl1 localizes as distinct foci on meiotic chromosomes throughout meiotic prophase I in budding yeast and localizes to kinetochores by metaphase I (Monje-Casas et al. 2007; Yu and Koshland 2007). In mouse spermatocytes, another chromosomal passenger complex protein, INCENP, shows confluent colocalization with the central element component SYCP1 from zygotene to pachytene, before relocating to the centromeres at late pachytene (Parra et al. 2003, 2009). Although Aurora B kinase staining is detected only from diplotene onward (Parra et al. 2003), inhibition of Aurora B kinase with ZM447439 blocks dissociation of the lateral element component SYCP3. However, the dissociation of SYCP1, the central element, is not affected (Sun and Handel 2008).

Here, we investigate Ipl1's role in SC assembly and disassembly in budding yeast. Cytological and biochemical observations show that Ipl1 promotes the timely disassembly of the SC prior to diplotene and the G2/M transition. Crossover levels are not decreased in the *ipl1* mutant and cell cycle progression occurs with similar kinetics to the wild-type strain. These observations support the conclusion that SC disassembly, promoted by Ipl1, occurs in parallel with cell cycle progression and maturation of crossover products.

Results

SC disassembly and Zip1 degradation are delayed in ipl1-mn mutants.

Ipl1 is expressed throughout meiosis, although at a lower level compared with mitotic cells (Monje-Casas et al. 2007). To investigate whether Ipl1 affects SC disassembly, we used a strain where *IPL1* expression is regulated by the *CLB2* promoter, which is down-regulated during meiosis (*P_{CLB2}-IPL1*, or *ipl1-meiotic null*) (Monje-Casas et al. 2007). 3HA-Ipl1 levels decrease in the *ipl1-mn* strain upon entry into meiosis and Ipl1 kinase activity becomes

undetectable compared with the wild-type strain, as assessed by phosphorylation of H3 Ser10 during meiotic prophase I (Supplemental Fig. S1). Using the *ipl1-mn* and an isogenic wild-type strain, we assessed surface spread nuclei for Zip1 staining (Fig. 1A). In the wild type, foci of Zip1 (leptotene) were observed in one-third of the nuclei by 2 h, short linear structures of Zip1 interspersed with Zip1 foci ("dot-linear," zygotene) appeared around 4 h, and nuclei consisting entirely of linear Zip1 staining (pachytene) were observed from 5 h. In the *ipl1-mn* mutant, SC assembly occurred similarly (Fig. 1B,C), although we observed a delay in the appearance of nuclei with punctate Zip1 from the 2- to 3-h time point in the *ipl1-mn* mutant. Subsequent analyses showed that other meiotic events, such as DNA replication, DSB formation, and the nuclear divisions, were also delayed (see below), suggesting a modest defect in the initiation of meiosis, as reported previously (Monje-Casas et al. 2007). Thus, the slight delay in SC assembly is most likely due to a general delay in meiotic progression.

Accumulation of Zip1 aggregates (polycomplexes, PCs), are observed in a large proportion of nuclei from mutants with defective SC assembly (Roeder 1997; Zickler and Kleckner 1999). However, in both the wild-type and

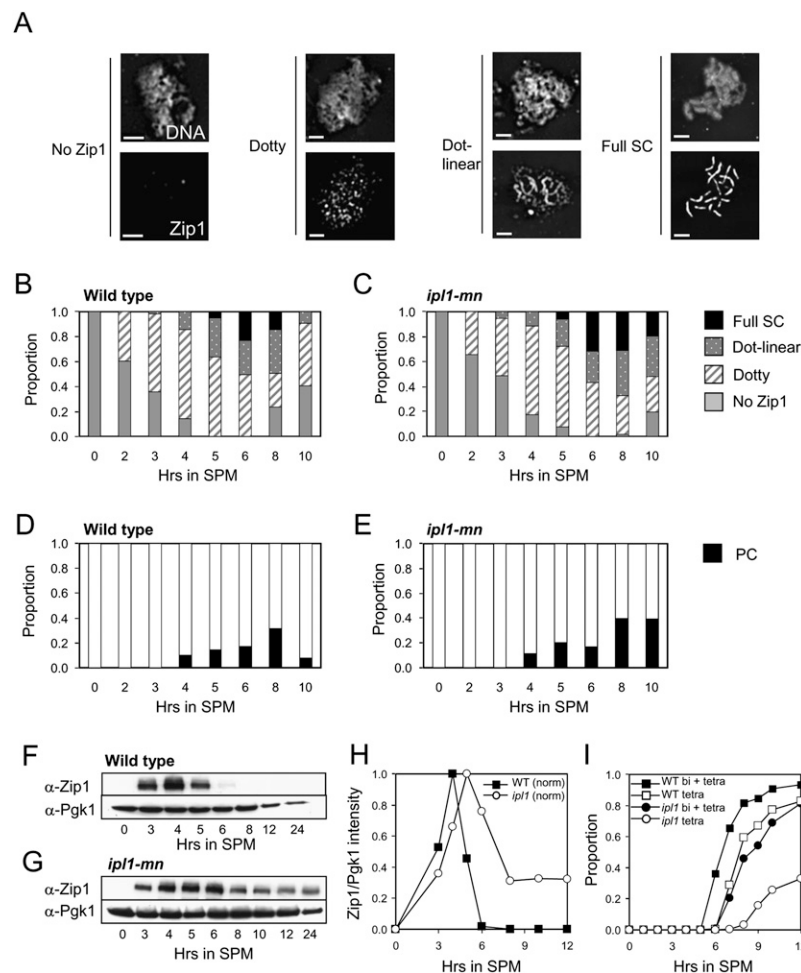


Figure 1. SC disassembly and Zip1 degradation are delayed in *ipl1-mn* mutants. (A) Examples of Zip1 staining. (B,C) Proportion of nuclei with dotty or punctate, dot-linear, linear Zip1, and no staining in wild type (Y940, B) and *ipl1-mn* (Y1206, C). More than 50 nuclei were scored for each time point. (D,E) Proportion of nuclei with Zip1 aggregates (PC). (F,G) Western blot analysis of Zip1 appearance and disappearance in wild-type (F) and *ipl1-mn* (G) cells in a separate experiment. (H) Ratios of Zip1/Pgk1 levels normalized to the maximum Zip1/Pgk1 ratio (Materials and Methods). (I) Completion of the two meiotic nuclear divisions. Cells were assessed whether they were binucleate (meiosis I) or tetranucleate (meiosis II). In a proportion of the *ipl1-mn* nuclei, the DNA looked "stretched," suggesting that metaphase I–anaphase I transition was delayed (Monje-Casas et al. 2007). Subsequent analysis demonstrated that these "stretched" nuclei contained long metaphase I/early anaphase I spindles (Supplemental Fig. S2). Nevertheless, by 24 h, 99% of wild-type and 88% of *ipl1-mn* cells had completed at least one meiotic nuclear division.

ipl1-mn mutant, PCs were observed in only ~10%–20% of nuclei during early time points (2–5 h in sporulation medium, SPM) (Fig. 1D,E), indicating that SC assembly occurred normally.

In the wild type, the proportion of nuclei with entirely linear Zip1 increased to ~25% by 6 h before decreasing in frequency at 8 h. By 10 h, no such nuclei were detected (Fig. 1B). This loss is interpreted as exit from the pachytene stages and coincides with an increase in surface spread nuclei containing PCs of Zip1 (~35%). These observations are consistent with electron micrograph studies that show a single dense body stained in nuclei with separated SPBs, indicative of diplotene (Zickler and Olson 1975). We find that the Zip1 PCs are often juxtaposed to duplicated SPBs lying side-by-side (Fig. 2A, “diffuse stage”).

The decrease in the frequency of nuclei with full SCs by 8 h was not observed in the *ipl1-mn* mutant. Even at 10 h, ~20% of nuclei displayed linear Zip1 staining. Thus, Zip1 is observed as linear stretches for an extended period in the absence of Ipl1. One interpretation of this observation is that the pachytene period is extended.

An extended persistence of nuclei with mature SC could be indicative of pachytene arrest or a delay in SC disassembly. To distinguish between the two possibilities, we followed the degradation of Zip1 and the nuclear divisions. Upon pachytene exit, Zip1 degradation occurred rapidly in the wild-type strain as cells entered the two nuclear divisions (Fig. 1F–I). In the *ipl1-mn* mutant, some Zip1 protein was degraded; however, a substantial fraction (~30%–40%) was retained (from 8 to 24 h), even as the number of cells that had completed at least one meiotic nuclear division doubled in the same time period (Fig. 1I). These observations are consistent with the hypothesis that SC disassembly is delayed.

Finally, in the *ipl1-mn* mutant, we noticed that a significant proportion of binucleate cells (~20%–30%) appeared “stretched,” rather than having properly divided their DNA mass. We examined this further and observed that these cells contained long metaphase I/early anaphase I spindles (Supplemental Fig. S2). This is consistent with previous observations that the transition from short metaphase I to anaphase I spindles is delayed in the *ipl1-mn* mutant (Monje-Casas et al. 2007). A similar phenomenon was also observed at metaphase II/anaphase II

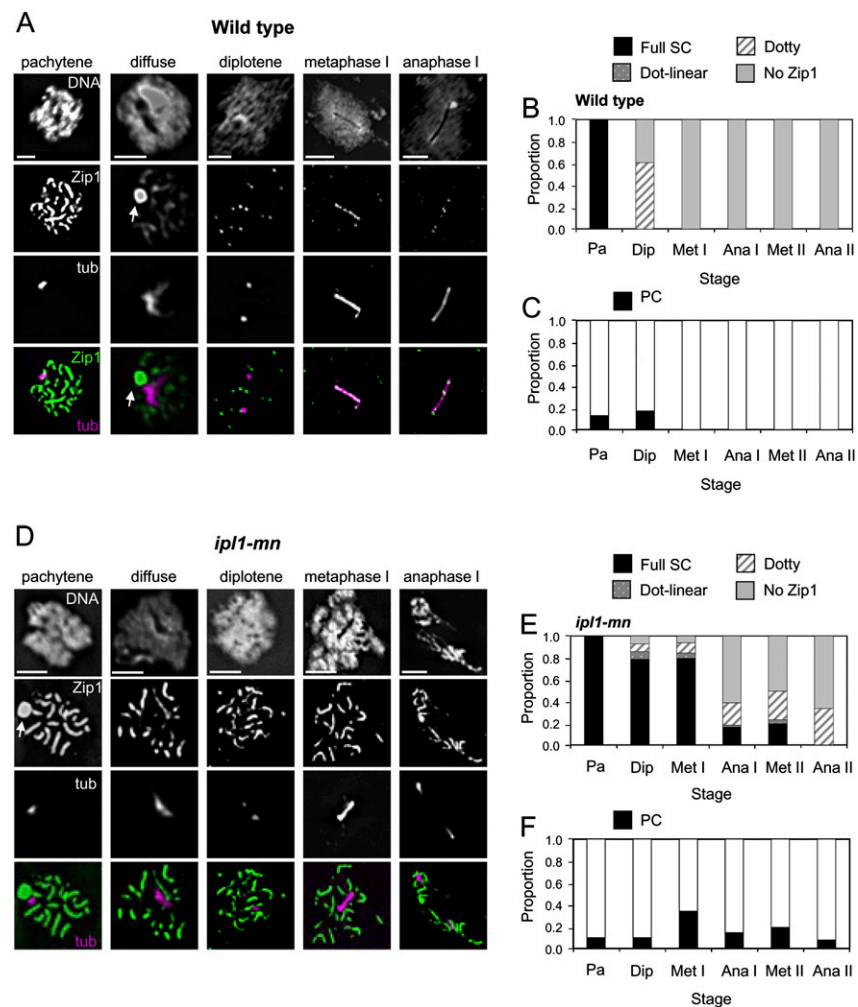


Figure 2. SC disassembly and cell cycle progression are decoupled in *ipl1-mn* cells. Surface spread nuclei were stained for Zip1 (green), tubulin (magenta), and DNA. (A,D) Examples of wild-type (Y1381) and *ipl1-mn* (Y1669) nuclei at pachytene, diffuse, diplotene, metaphase I, and anaphase I stages. The arrows indicate PCs of Zip1. (tub) Tubulin. Bars, 2 μ m. (B,E) Quantification of Zip1 staining based on cell cycle stage (as determined by tubulin staining). (Pa) Pachytene (single tubulin focus); (Dip) diplotene (two tubulin foci); (Met I) metaphase I (single short thick spindle); (Ana I) anaphase I (single elongated spindle); (Met II) metaphase II (two short thick spindles); (Ana II) anaphase II (two elongated spindles). (C,F) Proportion of nuclei with PC. For each stage, 30–50 nuclei were scored.

(Supplemental Fig. S2). The stretched nuclear phenotype is not alleviated by deletion of *ZIP1*, since an *ipl1-mn zip1* double mutant also displayed a stretched nuclear phenotype. If anything, both the double and the *zip1* single mutant displayed a more pronounced phenotype (Supplemental Fig. S3).

SC disassembly and spindle formation are decoupled in ipl1-mn

If SC disassembly is incomplete or delayed, but the meiotic nuclear divisions occur with only a short delay relative to wild type (Fig. 1I; Supplemental Fig. S2), then SC disassembly and the cell cycle must be decoupled. This hypothesis predicts that cells should be observed in which the SCs are still assembled despite progression of spindle behavior to diplotene or beyond. To test whether this was the case, we stained surface spread nuclei with antibodies against both tubulin and Zip1 (Fig. 2). In wild type, the SCs had disassembled by diplotene and only foci of Zip1 were present (Fig. 2A,B). We showed recently that these foci localize to centromeres (L Newnham, P Jordan, B Rockmill, S Roeder, and E Hoffmann, in prep.). By metaphase I, Zip1 was observed predominantly on the metaphase I spindle (Fig. 2A,B).

In contrast to the wild-type cells, ~80% of *ipl1-mn* mutant nuclei with separated SPBs or metaphase I spindles displayed linear Zip1 staining (Fig. 2D,E). Since SPB separation and metaphase I spindle formation depend on CDK1-M activity (Xu et al. 1995; Sourirajan and Lichten 2008), these results support the conclusion that SC disassembly and cell cycle progression have become decoupled in the absence of Ipl1.

Meiotic prophase I is not contracted in ipl1-mn

The decoupling of SC disassembly and spindle behavior shows that SC disassembly occurs in parallel with cell cycle control. The delayed SC disassembly could be due to Ipl1 regulating the timing of meiotic prophase I or SC disassembly per se. To distinguish between these possibilities, we assessed the progression of the meiotic cell cycle in the *ipl1-mn* mutant by analyzing the kinetics of premeiotic S-phase completion and SPB separation, the last stage of meiotic prophase, as well as the expression of two B-type cyclins—the meiosis I-specific Clb1 and meiosis II-specific Clb3 (Carlile and Amon 2008). Although premeiotic S phase was initiated at time points similar to the wild-type strain (2–3 h), completion was delayed by ~30–60 min (Fig. 3A; data not shown). SPB separation was similarly delayed by ~30–60 min compared with the wild type (Fig. 3B), indicating that meiotic prophase I is not contracted in the *ipl1-mn* mutant, consistent with previous observations (Monje-Casas et al. 2007).

To determine whether a contraction in meiotic prophase I is sufficient to cause a SC disassembly phenotype, we assessed SC disassembly in a *mad3Δ* mutant, which halves the duration of meiotic prophase I (Cheslock et al. 2005). Nevertheless, SC disassembly occurs prior to metaphase I (zero of 30 metaphase I nuclei contained

linear SCs) (data not shown), consistent with the interpretation that contraction of prophase I is insufficient to decouple SC disassembly from cell cycle progression.

Finally, we assessed whether the expression of B-type cyclins was affected in the *ipl1-mn* mutant. To this end, we used a strain where Ndt80 expression was shut off, but was inducible by addition of β -estradiol (*NDT80-IN*) (Benjamin et al. 2003; Carlile and Amon 2008). Using this system, >80% of nuclei from both the wild-type and the *ipl1-mn* strains showed full SCs prior to release (data not shown). Nevertheless, Zip1 disappearance and SC breakdown were consistently delayed in *ipl1-mn* relative to wild type upon Ndt80 expression. Expression of the meiosis I-specific B-type cyclin Clb1-13Myc (Carlile and Amon 2008) was similar in the *ipl1-mn* and wild-type strains (Fig. 3C). Thus, the transition from meiotic prophase to the first meiotic division appeared to occur normally in the *ipl1-mn* mutant.

Clb3-13Myc expression is confined to meiosis II (Carlile and Amon 2008) and occurred with similar timing in the *ipl1-mn* and the wild-type strains (Fig. 3D), despite the finding that anaphase I spindles appear to persist (Monje-Casas et al. 2007) and that a significant proportion of *ipl1-mn* nuclei showed progression defects from metaphase I–anaphase I (“stretched” DNA phenotype) (Supplemental Fig. S4). However, the nuclear division profile in the Clb3-13Myc time course shows that the initiation of the second nuclear division, which gives rise to a high proportion of stretched nuclei in the *ipl1-mn*, nevertheless occurred with similar timing as in the wild-type strain (Supplemental Fig. S4). Thus, although the completion of both meiotic nuclear divisions was delayed in the *ipl1-mn* strain, initiation thereof and Clb3-13Myc expression occurred with timing similar to the wild-type strain.

Ipl1 kinase activity is important for SC disassembly

Ipl1 is an ATP-dependent kinase whose ATP-binding domain can be mutated to accommodate a larger nucleoside analog that inhibits its activity (*ipl1-M181G,T244A*, aka *ipl1-as5*) (Pinsky et al. 2006). Inhibitors include 1-NA-PP1 (1-[1,1-dimethylethyl]-3-[1-naphthalenyl]-1H-pyrazolo[3, 4-d]pyrimidin-4-amine) and generally do not affect other, unmodified ATP-dependent kinases (Bishop et al. 2000). We inhibited *ipl1-as5* cells with 1-NA-PP1 after SC assembly, but prior to the onset of SC disassembly, by arresting cells in pachytene (at 5.5 h) and then releasing cells using the *NDT80-IN* (6 h). The 1-NA-PP1-treated *ipl1-as5* cells recapitulated the *ipl1-mn* phenotype (Supplemental Fig. S5), whereas mock treatment (DMSO alone) had no effect (data not shown). A slightly milder effect of the *ipl1-as5* allele compared with *ipl1-mn* was observed; this is likely due to a subset of cells not being exposed to sufficient levels of inhibitor. In diplotene and metaphase I nuclei, 32% and 30% contained full SCs compared with 40% in *ipl1-mn* (data not shown). These observations support the conclusion that Ipl1 promotes de facto SC disassembly and that it requires its kinase activity to do so. Whether or not Ipl1 phosphorylates

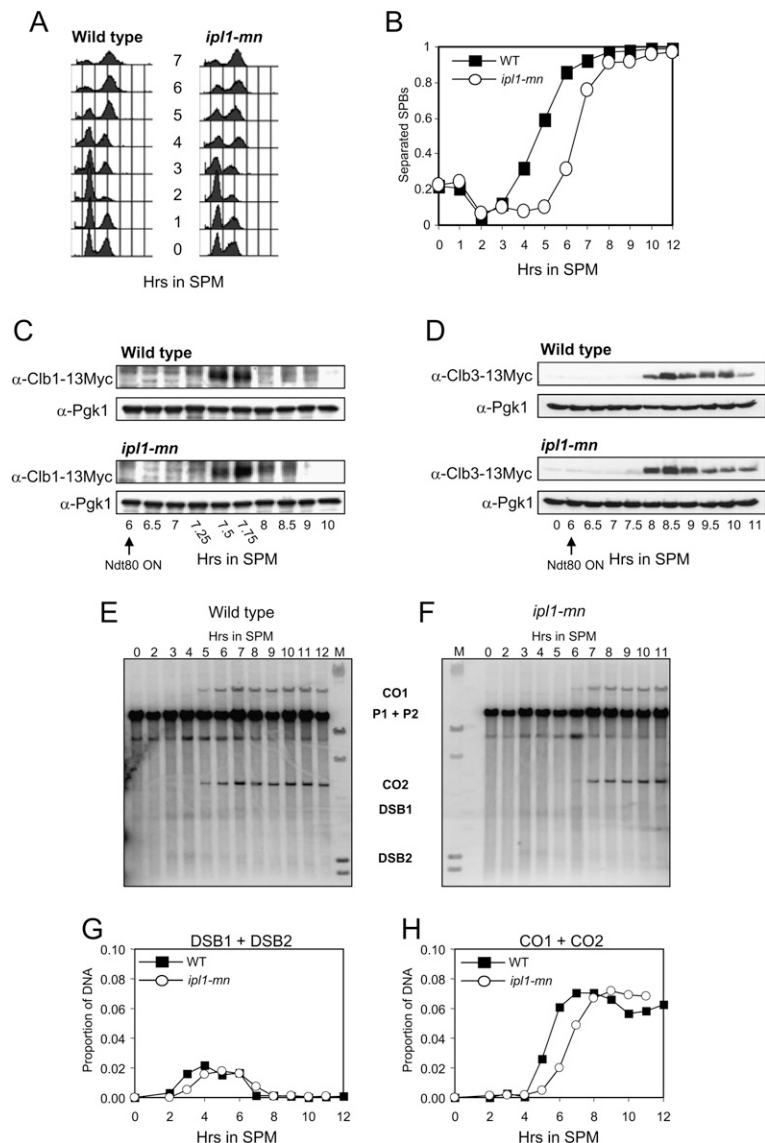


Figure 3. Cell cycle progression and crossover formation are relatively normal in the *ipl1-mn* mutant. (A) FACS analysis of premeiotic S-phase completion in wild type (Y940, left panel) and *ipl1-mn* (Y1206, right panel). (B) Proportion of cells with separated SPBs. More than 200 cells were scored per time point. (C,D) Expression of B-type cyclins and nuclear divisions in *NDT80-IN* wild-type and *ipl1-mn* strains. (C) Expression levels of Clb1-13Myc, a meiosis I-specific B-type cyclin in wild type (Y1661, top panels) and *ipl1-mn* (Y1663, bottom panels). (D) Expression levels of Clb3-13Myc, a meiosis II-specific B-type cyclin in wild type (Y1581, top panels) and *ipl1-mn* (Y1582, bottom panels). Pgk1 was used as loading control. The arrow indicates release from pachytene arrest by induction of Ndt80. (E,F) Southern blots of crossover formation at the *URA3-ARG4* hot spot (Supplemental Fig. S7) in wild type (Y940, E) and *ipl1-mn* (Y1206, F). (P1, P2) Parental DNA molecules; (CO1, CO2) crossover products; (DSB1, DSB2) DSB products. Molecular weight markers (M) are a *Hin*DIII digest of bacteriophage λ DNA (23.1, 9.4, 6.6, 4.4, 2.3, and 2.2 kb from top to bottom). (G,H) Quantification of DSBs and crossovers.

Zip1 remains to be determined. Although Zip1 is a phosphoprotein, its migration on one-dimensional PAGE remains unaffected in *ipl1-mn* mutants (Supplemental Fig. S6). We do note that simultaneous mutation of three potential Ipl1 phosphorylation consensus sites in Zip1 [ZIP1-T273A, S274A, D381A, T382A, S511A, and T512A] affects neither SC assembly nor disassembly (data not shown).

The DSB-crossover transition occurs normally in the *ipl1-mn* mutant

The SC disassembly defect observed in the *ipl1-mn* mutant allowed us to address whether SC disassembly is a prerequisite for crossover formation. To this end, we analyzed meiotic crossing over at the *URA3-ARG4* recombination hot spot inserted at the *LEU2* (parent 1, P1) and *HIS4* (parent 2, P2) loci, respectively, on the two parental Chromosomes III (Allers and Lichten 2001)

(Supplemental Fig. S7). DSBs were formed between 2 and 3 h in the wild-type strain (Fig. 3E,G) and were delayed ~60 min in the *ipl1-mn* mutant (Fig. 3F,G). This is consistent with the similar delay in the completion of premeiotic S phase, since replication timing determines the onset of DSB formation in budding yeast (Borde et al. 2000). Crossovers were formed between 4 and 5 h in the wild-type and 5 and 6 h in *ipl1-mn* strain (Fig. 3H). Thus, the time between DSB and crossover formation (~2 h) was similar in both strains. Furthermore, final crossover levels were also similar in the *ipl1-mn* mutant compared with the wild-type strain (~7%) (Fig. 3H). Finally, analysis of the levels of crossover and noncrossover products also failed to show any differences between the wild-type and the *ipl1-mn* mutant, except for the ~60-min delay (Supplemental Fig. S8). These observations support the conclusion that crossover formation and DSB repair, more generally, are not affected in the *ipl1-mn* mutant. Thus,

the delayed and incomplete SC disassembly phenotype does not cause adverse effects on crossover formation.

*IPL1 promotes SC disassembly in *cdc5-mn* and *CDC5-IN* cells*

Recently, Cdc5 Polo-like kinase has been implicated in pachytene exit, promoting Holliday junction resolution and SC disassembly. The period in which pachytene nuclei are observed is increased in *cdc5-mn* mutants, similar to *ipl1-mn* cells (Brar et al. 2009). When analyzed by tubulin staining, SC disassembly is less affected in a *cdc5-mn* mutant compared with *ipl1-mn*. Fewer than 20% of diplotene nuclei contained full SCs, and by metaphase I, this was further decreased (4%) (Fig. 4A,C). In the majority of nuclei at diplotene or beyond, Zip1 was often present as foci in the *cdc5-mn* mutant (Fig. 4A). These foci were more numerous than in wild type, where

Zip1 was retained at centromeres after pachytene exit (data not shown).

SC disassembly was similarly delayed in the *ipl1-mn cdc5-mn* double mutant compared with the *ipl1-mn* single mutant (Fig. 4B,D). Thirty-two percent of metaphase I nuclei contained full SCs and another 63% contained dot-linear Zip1 staining compared with 49% full SCs and 48% dot-linear in *ipl1-mn* (data not shown), and with 4% full SCs and 36% dot-linear in the *cdc5-mn* mutant (Fig. 4C). Furthermore, the dot-linear staining pattern of *ipl1-mn* and the *cdc5-mn ipl1-mn* double mutant contained more lines of Zip1 compared with *cdc5-mn*. Thus, 97% and 95% of metaphase I nuclei contained significant stretches of Zip1 in the *ipl1-mn* and *cdc5-mn ipl1-mn* mutants. These observations indicate that Ipl1 promotes SC disassembly in *cdc5-mn* mutants. Importantly, these experiments were carried out in the *NDT80-IN* background. We did not observe any progression

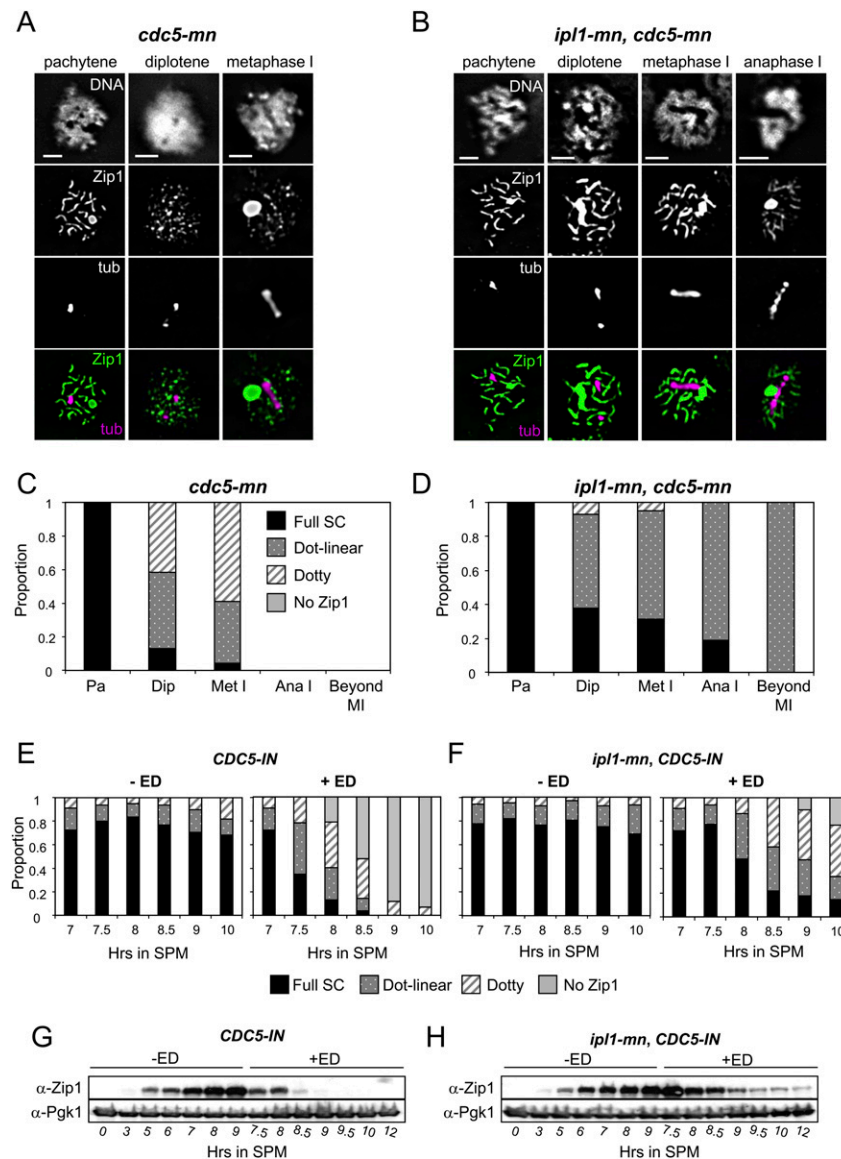


Figure 4. Ipl1 promotes SC disassembly in *cdc5-mn* and *CDC5-IN* cells. Examples of nuclei and quantification of Zip1 staining from *cdc5-mn* (Y1657, A,C) and *cdc5-mn ipl1-mn* (Y2030, B,D) mutants. (E,F) Analysis of Zip1 SC disassembly in a time course using a *ndt80Δ* strain with inducible *CDC5* (*CDC5-IN*, Y2262, E) expression by addition to β-estradiol (+ED, at 7 h) and in an *ndt80Δ CDC5-IN ipl1-mn* strain (Y2263, F). At least 100 nuclei were scored for each stage. Zip1 protein levels are shown for *ndt80Δ CDC5-IN* (G) and *ndt80Δ CDC5-IN ipl1-mn* (H) strains.

beyond pachytene in *cdc5-mn NDT80-IN* cells, where *NDT80* expression was not induced by addition of β -estradiol ($-\text{ED}$) (Fig. 4E; data not shown). This suggests that *CDC5* activity is extremely low, if present at all, in these cells.

Finally, expression of *CDC5* in an *ndt80* mutant (*CDC5-IN*) is sufficient to trigger SC disassembly (Fig. 4E,G; Sourirajan and Lichten 2008). We find that the *ipl1-mn* allele delays SC disassembly in the *CDC5-IN* strain, suggesting that *Ipl1* is important for SC disassembly regulated by *Cdc5*.

Zip1 degradation is inhibited in MG-132-treated cells

In wild type, *Zip1* dissociation from meiotic chromosomes and its degradation occurred with similar timing

after pachytene release (Fig. 5A,B). To address whether the two can be decoupled and whether the degradation of *Zip1* depends on the 26S proteasome, we treated *NDT80-IN pdr5 Δ* cells with the 26S proteasome inhibitor MG-132 (*Pdr5* is a multidrug transporter that pumps out MG-132 and is dispensable for meiosis) (Carlile and Amon 2008; data not shown). Cells were arrested at pachytene, and treated with 20 μM MG-132 or DMSO (5.5 h) before being released from arrest by induction of *Ndt80* expression via addition of β -estradiol (6 h, “*Ndt80 ON*”). Additional MG-132 or DMSO was added at 1-h intervals (6.5 and 7.5 h) before being washed out (8.5 h) (Fig. 5E,G). In both mock-treated and MG-132-treated *pdr5 Δ* cells, SC disassembly occurred with similar kinetics (Fig. 5F,H). Sixty minutes after *Ndt80* induction, only $\sim 30\%$ of nuclei showed full SCs, and this proportion decreased

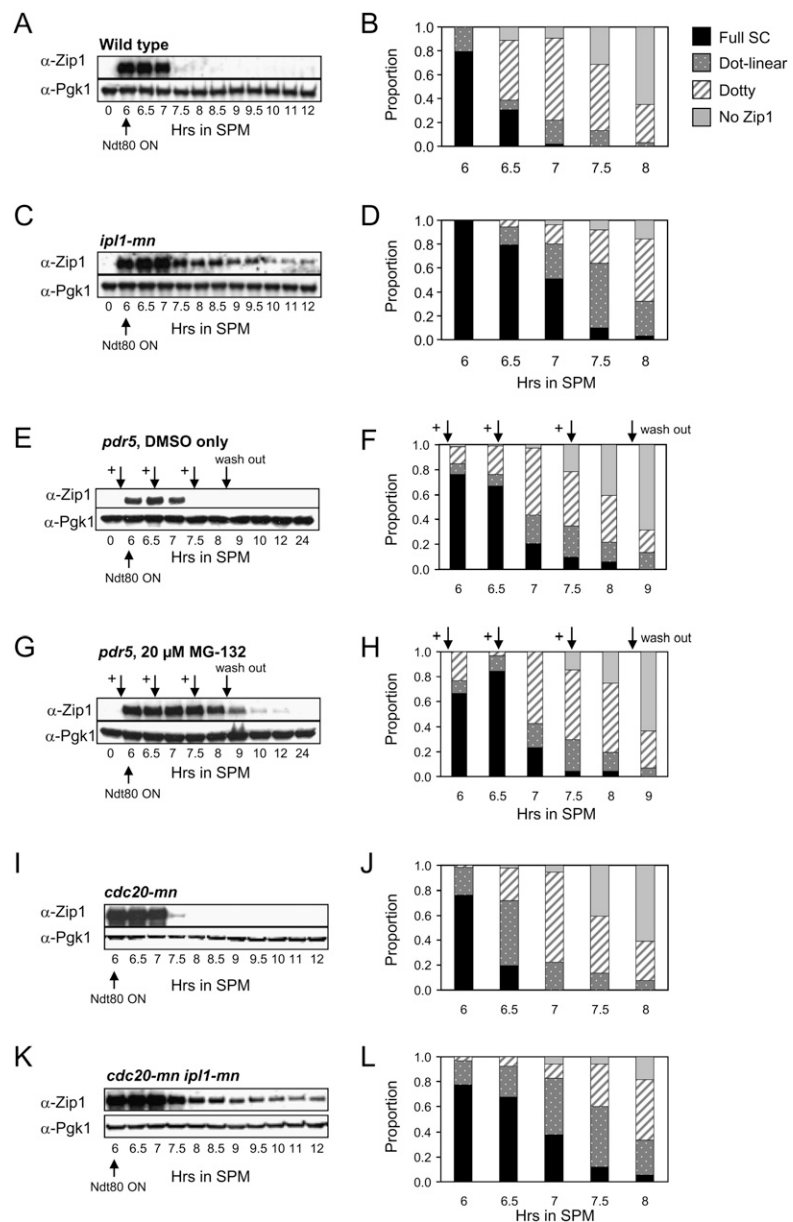


Figure 5. *Zip1* degradation and removal from meiotic chromosomes. Pachytene-arrested *NDT80-IN* cells were released by the addition of β -estradiol to induce *Ndt80* expression after 6 h in SPM (“*Ndt80 ON*,” indicated by the arrow), and *Zip1* and *Pgk1* protein levels as well as *Zip1* dissociation from meiotic chromosomes were determined. Arrest and release in wild type (Y1602, A,B) and *ipl1-mn* (Y1538, C,D). (E–H) Arrest-and-release experiment with *pdr5 Δ* cells (Y1553) treated with DMSO only (E,F) or 20 μM MG-132 (G,H) at the indicated time points (+). (I–L) SC disassembly and *Zip1* degradation after release from pachytene arrest in *cdc20-mn* (Y1656, I,J) and *cdc20-mn ipl1-mn* (Y1627, K,L). More than 50 nuclei were scored for each time point.

further with time, such that by 8 h, Zip1 had dissociated from the majority of meiotic chromosomes and no PCs were observed (data not shown). While Zip1 protein disappeared from mock-treated cells within 1.5 h of Ndt80 induction, Zip1 protein levels in MG-132-treated cells remained high until removal of MG-132 at 8.5 h. Zip1 protein levels then decreased (9 h) and eventually became undetectable (24 h) (Fig. 5E,G). Thus, in the presence of MG-132, Zip1 dissociated from meiotic chromosomes with normal timing but failed to be degraded. These observations show that Zip1 dissociation from chromosomes can occur independently of its degradation, as had been shown previously using *ndt80Δ* cells expressing the Cdc5 kinase (Fig. 4E,G; Sourirajan and Lichten 2008). Our data further indicate that Zip1 degradation is dependent on the 26S proteasome and that it can occur subsequent to Zip1 dissociation from chromosome cores.

Entry into anaphase I is not required for Zip1 degradation and SC disassembly in the ipl1-mn mutant

In the pachytene arrest-and-release experiments, Zip1 eventually dissociated from the chromosomes in the majority of nuclei in the *ipl1-mn* mutant (Fig. 5D). Consistent with this, only 20% of anaphase I nuclei, compared with 80% of metaphase I nuclei, contained Zip1 staining in the *ipl1-mn* mutant (Fig. 2E). Since metaphase I spindles can precociously elongate when homologous chromosomes fail to counteract the pulling forces (Visintin et al. 1998), we ascertained that Cdc14-13Myc, which is released from the nucleolus at the onset of anaphase I, was not sequestered in the cells containing anaphase I spindles (Supplemental Fig. S9).

To examine whether the SC dissociation in anaphase I nuclei of *ipl1-mn* cells requires entry into anaphase I, we examined Zip1 dissociation in cells lacking Cdc20, an activator of the APC important for anaphase I entry (Schwab et al. 1997; Visintin et al. 1997; Shirayama et al. 1998). *cdc20-mn* *NDT80-IN* or *cdc20-mn ipl1-mn* *NDT80-IN* cells formed metaphase I spindles with a single nucleus, and no anaphase I spindles were observed; however, more "stretched" nuclei were observed for *cdc20-mn ipl1-mn* (data not shown). Zip1 removal from meiotic chromosomes and Zip1 degradation occurred with normal timing after pachytene release of *cdc20-mn* cells (Fig. 5I,J), and *cdc20-mn ipl1-mn* double mutants removed and degraded Zip1 with the same timing as *ipl1-mn* single mutants (Fig. 5C,K,L). This indicates that the delayed dissociation of Zip1 in *ipl1-mn* mutants is independent of anaphase I entry, but may be correlated with the increased nuclear "stretching" observed.

Behavior of axial element components and Zip1-interacting partners

Smt3. Zip1 has been proposed to bind to sumoylated proteins associated with the lateral elements. In the wild type, Smt3 is observed along chromosome axes in pachytene nuclei (Fig. 6; Cheng et al. 2006; Hooker and Roeder 2006). By diplotene, Smt3 staining becomes less linear,

consistent with chromosomes becoming more diffuse. This behavior is different from that of Zip1 (Fig. 2A). As chromosomes condense again before metaphase I, Smt3 is found as thin, short stretches in ~40% of the nuclei and as short stretches interspersed with foci in another ~40% (Fig. 6A,B). By anaphase I, >70% of nuclei contain short stretches, interspersed with foci (Fig. 6A). No substantial aggregation of Smt3 was observed at any of the stages in the wild type (Fig. 6C). Consistent with Zip1 dissociating from meiotic chromosomes prior to Smt3, costaining of both proteins demonstrated that by diplotene and metaphase I, most Zip1 had been removed from chromosome arms despite Smt3 still being present (Fig. 6G; Supplemental Fig. S10).

In the *ipl1-mn* mutant, a significant proportion of diplotene nuclei contained linear Smt3 staining that was different from the short stretches observed in the wild type. Not only were the stretches longer, they also appeared thicker, as in pachytene (Fig. 6D). Thirty percent of nuclei contained aggregates of Smt3 (Fig. 6F). At metaphase I as well as anaphase I, ~80% of nuclei contained linear Smt3 staining, often with aggregates of Smt3 (Fig. 6E,F). Both the linear Smt3 as well as aggregates costained for Zip1 (Fig. 6H). These observations suggest altered association of Smt3 with chromosome cores at the G2/M transition in the *ipl1-mn* mutant.

Red1. Red1 is a component of axial elements and is found in discontinuous stretches, resembling "beads on a string" (Smith and Roeder 1997). We refer to these as short lines. Red1 dissociated from the meiotic chromosomes and was degraded with kinetics similar to Zip1 in the wild type (Fig. 7), although ~50% of nuclei showed discrete foci at metaphase I. Furthermore, in a subset of nuclei that contained metaphase I spindles (one-third), Red1 was observed to be juxtaposed with the spindle poles (Supplemental Fig. S11). Red1 protein levels were undetectable ~90 min after pachytene release (Fig. 7G), similar to Zip1 (Fig. 5A).

In the *ipl1-mn* mutant, Red1 was retained as foci at diplotene, and a proportion (~30%) of metaphase I nuclei contained short lines of Red1 (Fig. 7E). The majority of anaphase I nuclei did not contain any significant Red1 staining, and the one-third that did contained aggregates of Red1 (Fig. 7F). Instead of being associated with the spindle poles, these aggregates were found at the center of the anaphase I spindles (Supplemental Fig. S11). Overall, Red1 staining was significantly different from Smt3, although its behavior is also affected in the *ipl1-mn* mutant.

Red1 protein degradation occurred with only a slight delay in the *ipl1-mn* mutant (15–30 min) (Fig. 7H). Since Red1 is sumoylated (Cheng et al. 2006), Red1–Smt3 has been proposed to be a binding partner for Zip1. However, our cytological observations, supported by the Western blot analysis showing that Red1 protein levels are depleted more or less normally in *ipl1-mn* (Fig. 7), suggest that it is unlikely that sumoylated Red1 is the main binding partner for Zip1, at least after SC assembly.

Hop1. Like Red1 and Smt3, Hop1 and Rec8 are important for Zip1 localization to meiotic chromosomes. The

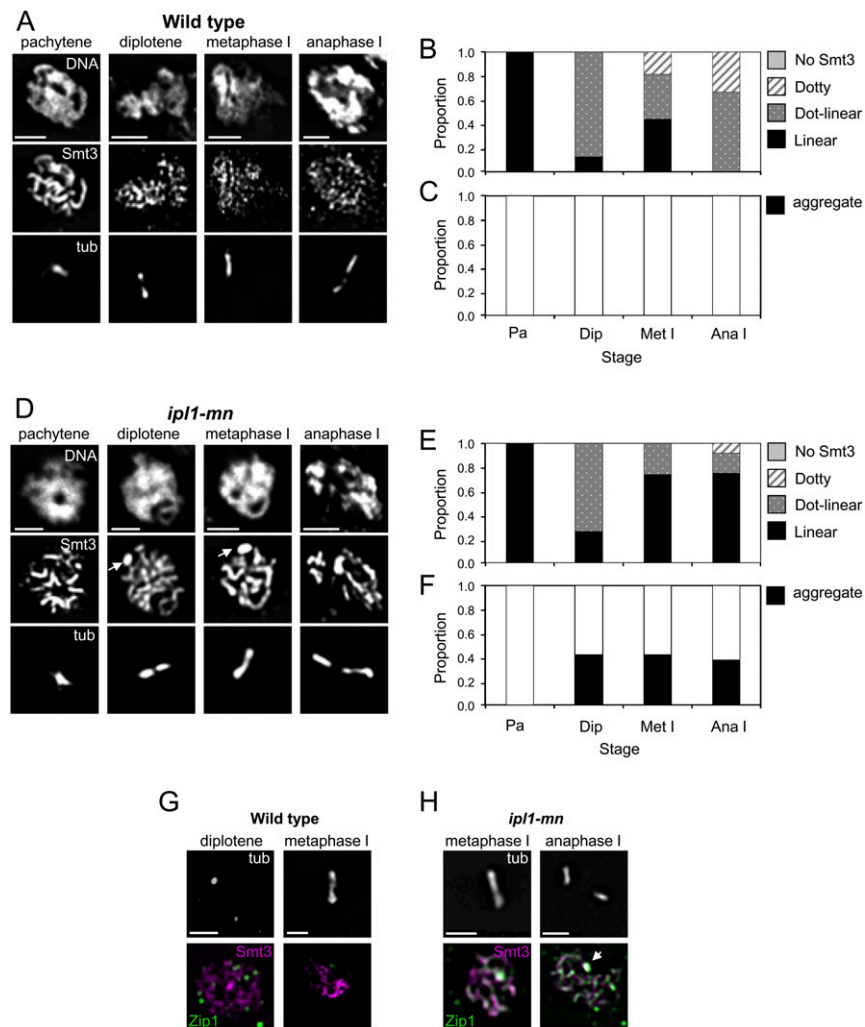


Figure 6. Aberrant association of Smt3/SUMO with meiotic chromosomes in the *ipl1-mn* mutant. (A–C) Examples of Smt3 staining at different stages of meiosis I (A) and quantification of Smt3 staining along chromosomes (B) as well as accumulation of aggregates (C) in the wild type. (D) Aberrant behavior of Smt3 in *ipl1-mn* at diplotene–anaphase I. (E,F) Quantification of nuclear staining (E) and aggregate formation (F). More than 50 nuclei were scored for each stage. (G,H) Dual staining of Zip1 (green) and Smt3 (magenta) in wild-type (diplotene and metaphase I, G) and in the *ipl1-mn* mutant (metaphase I and anaphase I, H). Arrows indicate aggregates of Smt3 and Zip1. (tub) Tubulin. Bars, 2 μ m. Single-channel images for Zip1 and Smt3 are shown in Supplemental Figure S10. Strains: Y940 (wild type) and Y1206 (*ipl1-mn*).

Hop1 staining pattern was similar in the wild type and the *ipl1-mn* mutant. Hop1 remained associated with the meiotic chromosomes after pachytene exit as multiple, distinct foci before appearing linear again in metaphase I and anaphase I (Supplemental Fig. S12). The persistence of Hop1 on meiotic chromosomes is consistent with findings that protein levels remain high, even as cells undergo the meiotic nuclear divisions (Carballo et al. 2008).

Rec8. Rec8 was present as linear structures until the metaphase–anaphase I transition in both the wild type and *ipl1-mn* mutant (data not shown), as reported previously (Yu and Koshland 2007). When we assessed spreads with two clearly separated nuclei (late anaphase I/telophase I), the characteristic pattern of Rec8 staining at the poles (presumably at centromeres) of the spindle was observed in the wild type. We also observed some Zip1 staining (Supplemental Fig. S13A). In contrast, in anaphase I/telophase I spreads with retained Zip1 and two clearly separated nuclei in the *ipl1-mn* mutant, Rec8 was also present (30 of 30 nuclei) and, generally, colocalized with Zip1 (Supplemental Fig. S13B).

Regulation of chromosome dynamics by Ipl1 does not require histone H3 phosphorylation on Ser10

Ipl1 phosphorylates histone H3 on Ser10 throughout meiosis I prophase (Supplemental Fig. S1; Monje-Casas et al. 2007). However, this phosphorylation event is dispensable for SC disassembly (Supplemental Fig. S14), indicating that other targets of Ipl1's kinase are important for SC disassembly.

Discussion

SC disassembly in budding yeast: Smt3 and Hop1 remain associated with meiotic chromosomes, while Zip1 localizes to the centromeres and Red1 dissociates at pachytene exit

Here, we investigated SC disassembly in budding yeast. At pachytene exit, both the central element component Zip1 and the lateral element component Red1 dissociate from the meiotic chromosomes and are degraded (Figs. 1, 2, 4, 6). Some Zip1 protein remains associated with centromeres and has been shown to be important for

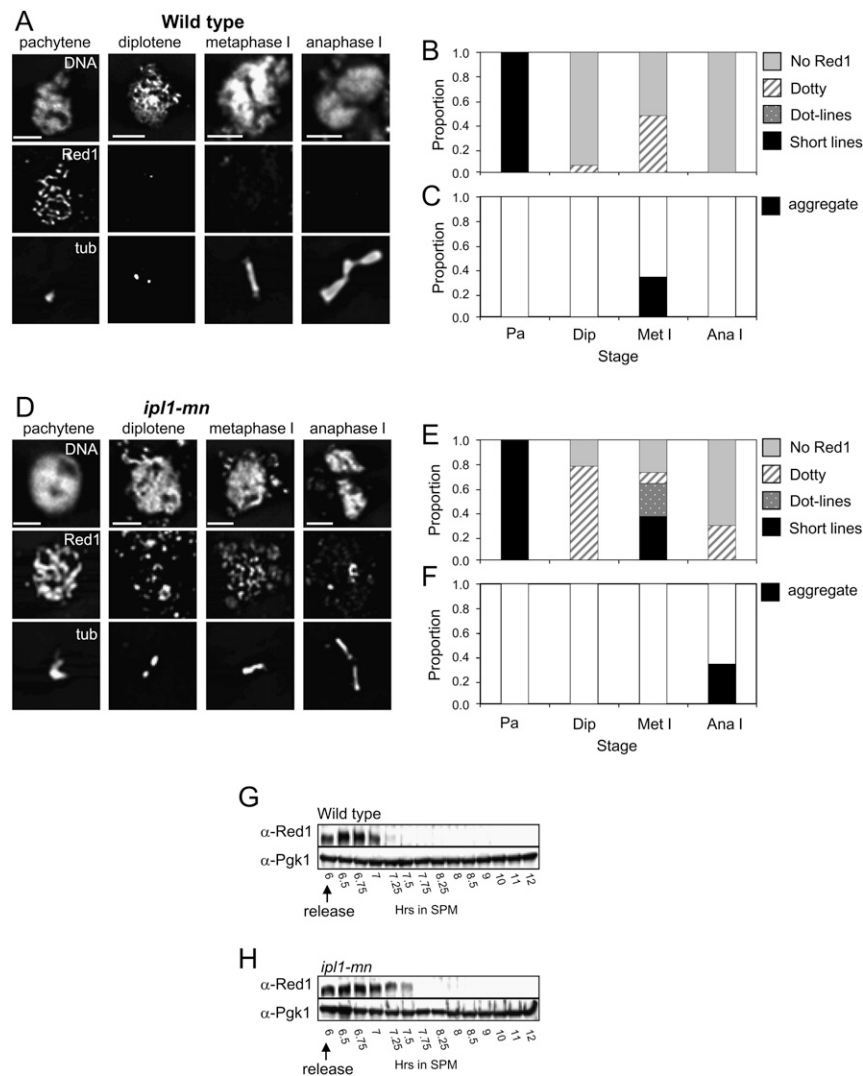


Figure 7. Red1 dissociation from meiotic chromosomes and its degradation are slightly delayed in the *ipl1-mn* mutant. Examples of Red1 staining in nuclei at various stages of meiosis I in wild type (A) and *ipl1-mn* (D). (tub) Tubulin. Bars, 2 μ m. Note that Red1 staining is not continuous along chromosomes, while Zip1 and Smt3 are. Quantification of Red1 staining of meiotic chromosomes and aggregate formation in wild type (B,C) and *ipl1-mn* (E,F). More than 50 nuclei were scored for each stage. Y940 (wild type) and Y1206 (*ipl1-mn*). (G,H) Western blot analysis of Red1 protein levels after pachytene release. Strains: Y1602 (wild type) and Y1538 (*ipl1-mn*).

nonexchange chromosome segregation (L Newnham, P Jordan, B Rockmill, S Roeder, and E Hoffmann, in prep.).

In contrast, Smt3/SUMO and Hop1 remain associated with the meiotic chromosomes as foci and short stretches even after the first meiotic division (Fig. 5; Supplemental Fig. S12). Thus, one protein known to interact with Zip1 (Smt3/SUMO) during SC assembly (Cheng et al. 2006) is retained after the dissociation of Zip1. This is different from the pattern observed during SC assembly, when Smt3 and Zip1 staining occur concomitantly (Hooker and Roeder 2006).

A role for Ipl1 in SC disassembly prior to its functions in M phase

Ipl1 has been found previously to have important roles in chromosome segregation during both meiosis I and meiosis II (Monje-Casas et al. 2007; Yu and Koshland 2007). Here, we identified a new function for Ipl1 in promoting SC disassembly (Figs. 1, 2), which precedes Ipl1's role in ensuring accurate chromosome segregation during M phase. Both components of the axial elements (Red1

and Smt3) as well as the central element (Zip1) display delayed dissociation from the meiotic chromosome cores in the *ipl1-mn* mutant. The delayed disassembly of the SC raises the possibility that the chromosome missegregation frequency of *ipl1-mn* mutants could be caused by its retention. This does not appear to be the case, since a homolog pair missegregates with similar frequencies in *ipl1-mn* and *ipl1-mn zip1* mutants (~80%) (Monje-Casas et al. 2007; data not shown).

Dissociation of central and lateral element components are delayed in *ipl1-mn*

Both disassembly of lateral element components as well as the central element component were affected in the *ipl1-mn* mutant. In the *ipl1-mn* mutant, Smt3 was observed as linear stretches after pachytene exit (Fig. 5). These, as well as Smt3 aggregates, colocalized with Zip1 in the *ipl1-mn* mutant (Fig. 5H), consistent with the model that Smt3 is a substrate of Zip1 (Cheng et al. 2007).

Red1, a lateral element component, showed slightly delayed dissociation from the chromosomes. However,

Red1 protein was eventually degraded (~30-min delay) (Fig. 6). Thus, the behavior of the central element component (Zip1) and its interacting partner (Smt3), as well as a lateral element component (Red1), are affected in the absence of Ipl1.

Two other lateral element components, Hop1 and Rec8, remain associated with the chromosome cores in wild type until the metaphase I–anaphase I transition and could be candidates for linking Zip1 to chromatin. For instance, the correlation and colocalization between Rec8 and Zip1 in anaphase I nuclei of *ipl1-mn* makes Rec8 a potential candidate (Supplemental Fig. S13B).

Given that Red1, Rec8, and Smt3 dissociation with the meiotic chromosomes is affected in the *ipl1-mn* mutant, we considered the possibility that Ipl1 might induce several changes to chromosome structure. One of the best-characterized targets of Ipl1 kinase is histone H3. Phosphorylation of histone H3 at Ser10 correlates with chromosome condensation in a variety of organisms, including budding yeast. During meiosis, Ser10 phosphorylation occurs at its highest levels between 3 and 6 h in meiosis (Supplemental Fig. S1), and chromosome spreads show costaining of phosphorylated histone H3 with Zip1 from zygotene to pachytene (Hsu et al. 2000). The role of this phosphorylation event, however, is unclear. Although *Tetrahymena* micronuclei mutated in Ser10 H3 show defects in chromosome condensation and meiotic transmission (Wei et al. 1998, 1999), budding yeast mutants lacking the target serine do not show any defects in mitotic or meiotic chromosome transmission (data not shown), consistent with previous observations (Hsu et al. 2000). We failed to observe any changes to SC disassembly in a strain expressing a histone H3 Ser10–Ala mutant protein (Supplemental Fig. S14). Thus, phosphorylation of histone H3 Ser10 is dispensable for SC disassembly in budding yeast. This conclusion is consistent with those made in studies of mouse spermatogenesis, where Aurora B kinase phosphorylation of histone H3 Ser10 occurs after the dissociation of the central element component SYCP1 (Sun and Handel 2008).

Our observations have some parallels with those made in *Drosophila* oocytes, where NHK-1 promotes SC disassembly of both central and lateral elements (Ivanovska et al. 2005). However, our observations differ from those made during mouse spermatogenesis. While inhibition of the Aurora kinases causes the retention of the lateral element component SYCP3, it does not prevent the dissociation of the central element SYCP1. Interestingly, in wild-type spermatogenesis, SYCP3 dissociates with similar characteristics to Zip1 in budding yeast, disassembling during late prophase I and remaining associated with the centromeric regions (Sun and Handel 2008).

Ipl1 ties SC disassembly to cell cycle progression and joint molecule resolution

Several cytological and biochemical observations support the conclusion that Ipl1/Aurora B kinase links SC disassembly to the G2/M transition in budding yeast. First, linear SCs are present in ~80% of metaphase I nuclei

from an *ipl1-mn* mutant (Fig. 2E), whereas no such nuclei were observed in wild type (Fig. 2B). Second, although S-phase completion is delayed ~30–60 min in the *ipl1-mn* mutant (Fig. 3A), subsequent cell cycle events, including SPB separation (Fig. 3B), are also similarly delayed in the *ipl1-mn* mutant (Fig. 3). This suggests that cell cycle progression occurs with similar kinetics as in wild type. Thus, the simplest explanation to account for the apparent decoupling of cell cycle progression (i.e., SPB separation and metaphase I spindle formation) and SC disassembly is that Ipl1 promotes the timely dissociation of Zip1 from the meiotic chromosomes at the end of pachytene.

One of the key events occurring at the end of pachytene is the resolution of joint molecules to crossovers. The *ipl1-mn* mutant showed normal levels of DSB formation and repair to both crossovers and noncrossovers (Fig. 3; Supplemental Fig. S8). Therefore, the retention of Zip1 on the chromosome does not appear to affect crossover formation. We conclude that Ipl1 ensures that full SC disassembly occurs at the same time as joint molecule resolution.

Four kinases regulate major events at the end of meiotic prophase

Our data show that Ipl1 links SC disassembly to both cell cycle progression and joint molecule resolution. In budding yeast, CDK1-M promotes SPB separation and the formation of metaphase I spindles. Neither is observed when the Cdc28 kinase subunit is inhibited, although SC disassembly and Zip1 degradation do occur (Sourirajan and Lichten 2008). Furthermore, joint molecule resolution is also not inhibited when Cdc28 is inactivated (Sourirajan and Lichten 2008). Rather, joint molecule resolution is promoted by Cdc5/Polo-like kinase. *cdc5-mn* mutants show high levels of joint molecule accumulation and low levels of crossovers, suggesting that Cdc5 activity is important for joint molecule resolution (Clyne et al. 2003). While Cdc5 expression is sufficient to promote full SC disassembly in *ndt80Δ* cells, full SC disassembly can occur in the absence of Cdc5 (Fig. 4). This is promoted by Ipl1, since the *ipl1-mn cdc5-mn* double mutant showed delayed SC disassembly, similar to *ipl1-mn*. Furthermore, *ipl1-mn* causes retention of SCs when *CDC5* expression is induced (Fig. 4). Our observations suggest that there are multiple mechanisms for SC disassembly. On the other hand, neither cell cycle progression nor joint molecule resolution is affected in the *ipl1-mn* mutant.

Collectively, these observations lead us to propose that four different kinases promote distinct events upon pachytene exit. CDK1-M ensures proper spindle behavior, Cdc5 promotes joint molecule resolution, Ipl1 stimulates SC disassembly, and Cdc7 as well as Cdc5 enforces mono-orientation of sister kinetochores (Clyne et al. 2003; Lee and Amon 2003; Lo et al. 2008; Matos et al. 2008).

It has been suggested previously that joint molecule resolution could promote timely SC disassembly (Sourirajan

and Lichten 2008). Although the *cdc5-mn* mutant displayed a relatively minor SC disassembly phenotype compared with *ipl1-mn* (Supplemental Table S1), the majority of nuclei at diplotene or beyond contained many foci of Zip1. These were more numerous than in wild type (data not shown), where Zip1 is retained at centromeres after pachytene exit (L Newnham, P Jordan, B Rockmill, S Roeder, and E Hoffmann, in prep.). It is possible that these Zip1 foci mark the sites of unresolved joint molecules. If so, then SC disassembly at sites of crossovers may be distinct from general removal of Zip1 from the arm regions. Currently, we do not have any cytological markers that allow us to identify (and therefore distinguish between) crossover products or joint molecules and correlate this transition with removal of Zip1 from the meiotic chromosomes on a per nucleus basis.

Materials and methods

Sporulation conditions

The strains used are listed in the Supplemental Material (Supplemental Table S2). Diploid cells were grown to saturation in YEPD (1% yeast extract, 2% bacto-peptone, 2% dextrose at pH 6.5), then inoculated at 5×10^6 cells per milliliter in SPS (0.05% yeast extract, 1% peptone, 0.17% YNB, 1% potassium acetate [KAc], 0.5% ammonium sulfate, 0.05 M potassium hydrogen phthalate at pH 5.5) and grown until a cell density of 5×10^7 cells per milliliter had been reached, before being resuspended in 1% potassium acetate, supplemented with 0.02% raffinose and 0.02% antifoam (pH 7.0) (Sigma, A8311). For S288c, cells were resuspended in YPA (1% yeast extract, 2% bacto-peptone, 2% KAc) and grown to 5×10^7 to 5.5×10^7 cells per milliliter before being induced to undergo meiosis in 2% KAc and 0.02% raffinose (pH 7.0).

Indirect immunofluorescence on whole cells

Indirect immunofluorescence was carried out by fixing 4–8 mL of cells ($\sim 2 \times 10^7$ cells per milliliter) in 4% formaldehyde for 15–60 min at room temperature. Cells were harvested and resuspended in 1 mL of IS buffer (1.2 M sorbitol, 100 mM K_2HPO_4 , 100 mM KH_2PO_4 , 100 mM $MgCl_2$). Cells were transferred to an Eppendorf tube and washed three times in IS buffer before being resuspended in 100 μ L of IS buffer containing 2 μ L of DTT (1 M) and 10 μ L of zymolyase 100T (10 mg/mL; Seiguku Corporation). The cells were incubated at 37°C until they had lost most of the cell wall and contained “rough” edges. Cells were washed with 100 μ L of PBS-N-N containing PBS + 150 mM NaCl and 50 mM Na_3PO_4 (pH 7.4), gently pelleted, and resuspended in 67 μ L of PBS-N-N + 0.1% NP40. After 30 min incubation at room temperature, 33 μ L of fetal bovine serum containing the appropriate primary antibodies were added, and cells were incubated for 4 h at room temperature and then overnight at 4°C. Cells were washed three times in 1 mL of PBS-N-N and resuspended in 50–100 μ L of mounting solution containing DAPI.

Meiotic surface spread nuclei

Surface spread nuclei were prepared as described (Chua and Roeder 1998). Primary antibodies were used in the following concentrations: rat α -tubulin (1:1000; Novus Biologicals), rabbit α -Zip1 (1:200; Eurogentec), goat α -Zip1 (1:100; Santa Cruz Biotechnologies, sc-15632), and rabbit α -Smt3 (1:100; Santa Cruz Biotechnologies, sc-28649). Secondary antibodies were obtained for Stratech and used 1:200. To identify meiotic nuclei at pachytene, we used highly condensed “worm-like” DNA and/or linear Zip1 staining. For subsequent stages, we used tubulin to assess SPB behavior. The diffuse stage was characterized by juxtaposed SPBs and diffuse DNA, whereas the diplotene stage was identified by a single body of DNA containing separated SPBs. For metaphase I, we used “club-like” spindles $\sim 2 \mu$ m, and for anaphase I we used spindles $>4 \mu$ m.

Western blot analysis

Proteins samples were prepared by post-alkaline extraction (Kushnirov 2000) or TCA extraction (Clyne et al. 2009). Cells from 2.5 mL of liquid culture were pelleted and resuspended in 100 μ L of 0.1 M NaOH, incubated for 5 min at room temperature, and resuspended in modified Laemmli sample buffer (0.06 M Tris-HCl at pH 6.8, 5% glycerol, 2% SDS, 4% β -mercaptoethanol, 0.0025% bromophenol blue). Protein samples were separated on SDS-PAGE gels, transferred to a nitrocellulose membrane, and probed with the appropriate primary antibodies, which included a goat α -Zip1 (1:2000; Santa Cruz Biotechnologies, sc-15632), rabbit α -Histone H3 (Millipore, 05-928, 1:2000), rabbit p-Histone H3 (Ser10)-R (1:2000; Santa Cruz Biotechnologies, sc-8656-R), rabbit α -Red1 (1:2000; Roeder Laboratories) (Smith and Roeder 1997), mouse 12CA5 α -HA (1:1000; CRUK), and α -3-PGK as a loading control (Molecular Probes, A-6457) at 1:10,000. The goat α -Zip1 IgG was detected with HRP-conjugated rabbit α -goat IgG (DakoCytomation, P0449), mouse α -3-PGK was detected with HRP-conjugated rabbit α -mouse IgG (DakoCytomation, P0260), and the antibodies raised in rabbit were detected with HRP-conjugated swine α -rabbit IgG (DakoCytomation, P0399). All secondary antibodies were diluted 1:2000. PerkinElmer Western Lightning Chemiluminescence Reagent and Fuji Super RX X-ray film were used for development. Protein levels were determined using ImageJ.

Purification of antibodies from serum

To purify IgGs from serum raised against Hop1 and Red1 proteins (gifts from Dr. Franz Klein and Professor G. Shirleen Roeder, respectively), Protein A beads/resin (Sigma) were washed once in PBS and resuspended in twice the volume of PBS (50% resin). One-hundred microliters of the 50% resin were incubated with 200 μ L of serum for 1 h at 4°C while rotating. This was followed by four washes in 2 mL of 0.1 \times PBS (diluted with water). IgGs were eluted in 0.2 M glycine (pH 2.5) (Fisher). All steps were carried out at 4°C or with samples on ice.

Jordan et al.

Southern blot analysis

Southern blotting was carried out as described (Allers and Lichten 2001). ImageJ (NIH) was used to quantify DNA levels.

Acknowledgments

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